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Electrochemical codeposition of gold particle–poly(2-(2-pyridyl) benzimidazole) hybrid film on glassy carbon electrode for the electrocatalytic oxidation of nitric oxide

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ABSTRACT

A nitric oxide (NO) sensor was developed by coating gold particles (AuPs) dispersed poly(2-(2-pyridyl) benzimidazole) (PPBZ) film on glassy carbon (GC) electrode by cyclic voltammetry. The PPBZ film modification enhances the electron transfer kinetics and the AuPs increase the surface area and electrocatalytic activities of GC electrode. A 4.04 fold increase in anodic current with 100 mV shifting of the peak potential towards less positive side was observed for the composite film modified electrode compared to that of bare GC electrode. The electron transfer coefficient, electron transfer rate constant, diffusion coefficient and catalytic rate constant for the electrooxidation of NO were investigated. The amperometric measurements revealed a linear range of detection from 1.7×10^{-8} to 2.0×10^{-6} M with a detection limit of 3.7 nM (S/N = 3) and sensitivity of 6.45 A M^{-1} cm^{-2}. The modified electrode possessed remarkable stability and reproducibility towards NO detection and was successfully applied for the detection of NO released from the living tissues.

1. Introduction

In 1987 it was confirmed that nitric oxide (NO) is the endothelium-derived relaxing factor (EDRF) [1] and it has been reported to mediate a large number of diverse pathological and physiological processes such as smooth platelet inhibition, muscle relaxation, immune regulation and penile erection [2,3]. It acts as a secondary messenger in signal pathways and an important regulatory molecule in the process of gene transcription and translation [4]. It also plays an important role in mediating signal transmission, apoptosis, vasoconstriction, gastrointestinal motility and immunity [5]. On the other hand, it associates with the mechanisms of many diseases, including hypertension, gastrelcosis, septic shock, central nervous system disorder, tumour, acute renal failure etc. [6–9]. A variety of approaches have been proposed for NO determination, including electron paramagnetic resonance (EPR) [10], spectrometry [11], chemiluminescence [12], UV–visible spectroscopy, fluorescence and electrochemical methods. Among these, electrochemical techniques is an effective tool for the rapid, direct, inexpensive and real-time analysis of biomolecules with high sensitivity and the only feasible method for NO detection in real samples [13].

Electropolymerization is a simple and effective method for the target selective modification of electrodes with specific matrices. Conducting polymer modified electrodes are widely used for electroanalysis due to its low ohmic drops and enhanced electron transfer rate constants for some electroactive species [14]. Benzimidazole and its derivatives can act as good conducting polymers [15] and also have significant role in antimicrobial [16,17], antibacterial [18], antiviral and antitumour activities [19]. Also, electrochemically deposited poly(2-mercaptobenzimidazole) was used for the recognition of pyrene [20]. 2-(2-pyridyl) benzimidazole (PBZ) was used as a fluorescent probe for hydration of nafion membranes [21] and for photoinduced proton-transfer processes [22].

Noble metal nanoparticles possess unique catalytic activities for both oxidation and reduction reactions. Gold particles (AuPs) have been extensively utilized in recent years due to its unique properties such as large surface to volume ratio, good conductivity, appreciable electrocatalytic ability and biocompatibility. The AuPs dispersed on various substrates have been reported, such as

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carbon paste electrode, self-assembled monolayer, conducting and non-conducting polymers [23–26]. Among these, conducting polymers are the suitable host matrices as they permit a facile electronic charge flow through the polymer matrix during electrochemical processes.

Several electrochemical sensors such as poly(*p*-phenylenevinylene) derivative (PPV) modified glassy carbon (GC) electrode [27], 4α-Co^{II}TAPc modified GC electrode [28], self-assembled trans-$[Fe(cyclam)(NCS)_2]^+$ complex ion modified Au electrode [29], AuNPs-ERGO modified GC electrode [30], nafion/multi-walled carbon nanotubes-chitosan-gold nanoparticles (Nafion/MWNTs-CS-AuNPs) modified GC electrode [31] etc. were reported for NO detection. The present work describes the sensitive and selective detection of NO using AuPs dispersed poly(2-(2-pyridyl) benzimidazole) (PPBZ) film modified GC electrode and its efficaciousness in the detection of NO released from the living tissues.

2. Experimental details

2.1. Reagents and solutions

2-(2-pyridyl) benzimidazole, $HAuCl_4{\cdot}xH_2O$ and D-(+)-glucose were purchased from Sigma Aldrich. L-Arginine (L-Arg), $NaNO_2$ and urea were purchased from Alfa aeser. $K_4[Fe(CN)_6]$, $K_3[Fe(CN)_6]$, KH_2PO_4, K_2HPO_4, NaCl, KCl, $CaCl_2$ and $MgCl_2$ were purchased from Merck. Phosphate buffer (PB) solution was prepared and the pH was adjusted using phosphoric acid. All the solutions were prepared in ultrapure water (Millipore-*Q*).

2.2. Apparatus

Electrochemical analyzer (CHI6043B, CH instruments, USA) coupled with a three electrode cell was used for electrochemical studies. A GC electrode of 3 mm diameter, a Pt wire and a saturated Ag/AgCl electrode were used as the working electrode, counter electrode and reference electrode, respectively. Atomic force microscope (AFM) analyses (non-contact mode) were performed on a XE-100 scanning probe microscope, Park systems, Japan and SEM study was conducted using Carl Zeiss Scanning electron microscope.

2.3. Procedure

2.3.1. Pretreatment

GC working electrode was polished with alumina powder having particle size 1, 0.3 and 0.05 μm successively and was then washed with double distilled water followed by sonication in millipore water for 10 min, before use. Further, the electrode was washed with ethanol and then with water.

2.3.2. Preparation of hybrid film modified electrode

The AuPs dispersed PPBZ film was electrochemically codeposited on GC electrode by cyclic voltammetry (CV). The monomer solutions of 10 mM PBZ and 1 mM $HAuCl_4$ prepared in 0.1 M H_2SO_4, were mixed in different ratios and was electrodeposited on GC electrode by applying potential between 1.5 and 0 V at a scan rate of 0.1 V s^{-1}. The number of potential scan required for the film was optimized by comparing the NO oxidation current responses from various film modified electrodes prepared by applying 5, 10, 15, 20 and 30 cycles. Simplex PPBZ and AuPs film modified GC electrodes were also fabricated by following the same procedure. All films prepared were rinsed with millipore water and dried before use. The bare and modified electrodes were characterized by recording voltammograms in an equimolar mixture of 1 mM each of $K_4[Fe(CN)_6]$ and $K_3[Fe(CN)_6]$ solution having 0.1 M KCl.

2.3.3. Electrochemical measurements

The electrochemical detection of NO was carried out in PB solution (pH 2.5) which was deaerated with high purity nitrogen (≥99.9%) gas prior to the experiment. Also, the whole cell was kept under inert atmosphere by passing nitrogen gas over the solution throughout the studies. Differential pulse voltammetry (DPV) measurements were carried out at an amplitude of 0.05 V, pulse width of 0.2 s and a pulse period of 0.5 s. Electrochemical impedance measurements were performed with the bare and modified electrodes as working electrodes at open circuit potential in the frequency range of 10 mHz to 100 kHz in 0.2 M PB solution of pH 2.5. Amperometric experiments were carried out in PB solution by successively spiking aliquots of sample from $NaNO_2$ stock solution at regular sample interval of 50 s.

2.3.4. Monitoring NO release from living organs

Goat and chicken liver samples were used for this study. The fresh samples were collected in a frozen container from a nearby butcher's shop, half an hour prior to the experiment. Each sample was then thoroughly rinsed with PB solution and was immersed in the buffer solution until used. The three electrode system was positioned into the PB solution near the liver tissue as close as possible and the amperometric signals were recorded with the addition of aliquots of L-Arg under unstirred condition.

3. Results and discussion

3.1. Morphology

The films were deposited on indium tin oxide (ITO) coated glass slides by following the procedure described in section 2.3.2 and the surface morphology was analyzed by AFM (Fig. S1) and SEM. A smooth surface was observed for bare ITO substrate (Fig. S1a), while the PPBZ film surface (Fig. S1b) possessed an irregular pattern. The AuPs were observed to be equally distributed on the ITO substrate (Fig. S1c) and the film thickness was found to be enhanced for the AuPs embedded PPBZ film (Fig. S1d). The SEM image of the composite film (Fig. S2) showed spherically shaped AuPs distributed on the PPBZ film with varying particle size of 150–200 nm.

3.2. Electrochemical characterization of the modified electrode

The consecutive voltammograms recorded during the electropolymerization of PPBZ possessed an anodic and cathodic peak at 0.32 and 0.28 V, respectively (Fig. S3A). The presence of pyridyl group, a lewis base, decreases the potential required for the oxidation of the monomer. The mechanism of electropolymerization is as shown in Scheme S1. During the potential cycling, the pyrrole nitrogen of PBZ is oxidized to form a radical cation and the single electron is delocalized over the benzene ring, in conjugation with it. The life time of the radical cation is increased by the delocalization process, subsequently increasing the probability of accomplishing radical–radical interactions. The C–C coupling of carbon localized odd electron resonance structure (IV), the more sterically favourable form, leads to the formation of a dimer. Further, the dimer undergoes oxidation and its coupling with resonance structure (IV) take places onto the available meta position versus pyrrole nitrogen atom. A series of such reactions occur to produce the so-called PPBZ. The cyclic voltammogram of the PPBZ film modified GC electrode in a monomer free PB solution showed polymer redox couple at 0.32 and 0.28 V (Fig. S3B) due to the doping and undoping of protons and anions in PPBZ film.